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Conditions in the static system favored pesticide degradation while the flowing system insured continuous pesticide exposure to the organisms. Pesticides were introduced in the systems at rates ranging from 0.1 to 100 ppm (static; adsorbed to soil) and 0.1 to 100 ppb (flowing; directly into water). Organisms included daphnids (Daphnia magna), algae (Oedogonium cardiacum), snails (Helisoma sp.), and mosquito fish (Gambusia affinis).

The total amount of ^{14}C-labeled PCNB (parent compound plus metabolites) accumulated by all organisms was about the same in each of the two ecosystems (for similar treatment rates). Pentachloronitrobenzene content in organisms reached equilibrium levels with water in 3 to 7 days and decreased 50 to 95% when placed in untreated water for 10 days. Variability between samples was high, primarily at the lower treatment rates and for algae. Analytical problems and precipatation difficulties in the flowing system were responsible for this variability. Simazine accumulation (parent compound plus metabolites, ^{14}C-labeled) by snails and fish was similar between systems for similar treatment rates, while algae and daphnids accumulated higher amounts of simazine in the flowing systems than in the static. Also, more degradation products were found in fish extracts from the static than in the flowing systems. Trifluralin was extensively degraded in the static system with very little accumulation of ^{14}C by the organisms. Further, little of the ^{14}C accumulated was trifluralin. Large amounts of trifluralin were accumulated by all organisms in the flowing systems, most of it being the parent compound. Also toxicity to algae and abnormal behavior responses of fish to the highest trifluralin level were observed.

The basic design of the two systems is sufficiently different that they cannot be routinely substituted for each other without first considering such factors as test compound degradability, likely mode of introduction into water and the type of toxicity data desired. However, the flowing system is more versatile in the types of data that can be obtained than either the static or simple flowing chronic testing systems, but requires a higher level of design, maintenance, and analytical input than the simpler systems.

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Figure 2. Flowing water model ecosystem. Tank is 63 x 32 x 41 cm and holds 60 liters of water.

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FOREWORD

There is a growing recognition that techniques are needed which are capable of measuring effects that are beyond the single species level of organization--yet reliable and readily useable techniques are few. One is faced with the need to choose the best system for measuring a desired effect, with little hard data on which to base such a decision.

This report provides such data for two model ecosystems containing the same organisms, but differing principally to the extent that one was a flow-through system and the other was a recirculating static system. Conclusions are presented on the relative advantages of each for testing chemicals of a range of degradability and for simulating distinctly different environmental situations.

J. David Yount
Deputy Director
Environmental Research Laboratory-Duluth

ABSTRACT

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To achieve this objective, a model ecosystem was designed and built that utilizes the continuous dosing, flow-through system routinely used for chronic fish toxicity testing in combination with the organisms used in static model ecosystem testing. A previously developed recirculating static model ecosystem (simulating a sediment or erosional pesticide source) was simultaneously used with the flowing water system (simulating an effluent pesticide source) to test the behavior of three pesticides (pentachloronitrobenzene (PCNB), simazine, and trifluralin).

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The basic design of the two systems is sufficiently different that they cannot be routinely substituted for each other without first considering such factors as test compound degradability, likely mode of introduction into water

and the type of toxicity data desired. However, the flowing system is more versatile in the types of data that can be obtained than either the static or simple flowing chronic testing systems, but requires a higher level of design, maintenance, and analytical input than the simpler systems.

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SECTION 1
INTRODUCTION

It is essential that we obtain as much information as possible about the fate and behavior of a chemical in and its effects on the aquatic environment before substantive quantities are released into natural waters. The model ecosystem, which can be built to simulate a desired part of the environment is a useful tool in obtaining this information. At present, fate and behavior studies are being carried out using static or single dose systems; flowing-water systems are being employed to obtain effects and toxicological data. The primary purpose of this project was to determine if characteristics of both flowing water and static systems could be incorporated to produce more comprehensive model ecosystems.

SECTION 2
CONCLUSIONS

The results of experiments where three pesticides were tested in two aquatic model ecosystems have indicated that static and flowing-water models are each valuable tools for determining the fate and behavior of pesticides in the aquatic environment, but cannot be routinely substituted for each other since differences in their basic design can result in very different results for the same compound. The static model ecosystems (that simulate a sediment or erosional pesticide source) used in these experiments provided excellent conditions for chemical and biological degradation while the flowing-water model ecosystem (that simulate an effluent pesticide source) provided continuous chemical exposure to the organisms and suppressed degradation. As a result trifluralin, which is subject to rapid degradation, showed a high level of accumulation and biological activity in the flowing system, but was rapidly degraded and not accumulated in the static system. The two other pesticides tested, PCNB and simazine, were less subject to degradation and as a result, far smaller differences between the systems were observed. Research on the behavior of pesticides (or other chemicals) in the aquatic environment may require use of more than one type of model ecosystem, depending on information being sought and properties of the compound. This study also showed that both the static and flowing systems can be used in toxicity and effects studies. However, if the compound is reasonably degradable, then the flowing system will give the most reliable information for the least amount of effort. Toxicity information on less degradable compounds can be obtained equally well from either system.

The cost and supply of radiolabeled compounds can be a determining factor in choosing between systems since the flowing system uses 10 or more times as much radiolabeled compound as the static.

SECTION 3
MATERIALS AND METHODS

Ecosystems

Two types of systems were designed and built for this study. The recirculating static ecosystem is a modification of static systems previously used in this laboratory (Figure 1). The system as shown has a total volume of 16 liters, with the small compartment occupying about one-fourth of the tank volume. The test chemical is introduced (adsorbed to soil) to the large compartment and carefully flooded with water to avoid suspending the soil. A water depth of 1 cm is maintained over the top of the glass partition. The percolator water pump is designed to maintain the same pesticide concentration on both sides of the glass partition and to transport fecal matter to the larger chamber where daphnids may use it as food. The screen-gate combination on top of the glass partition provides additional experimental flexibility to the model ecosystem. With the gate in place, daphnids are protected from predation which allows development of a stable population. With the gate removed, daphnids are subject to predation as they move into the fish compartment and thus complete the daphnid to fish food chain.

The flowing-water ecosystem (Figure 2) also includes a partitioned tank with the same organisms as in the static, but receives the test chemicals in the incoming water. The chemical is introduced in a proportional diluter, similar to that described by Mount and Brungs (1967). Flow rate was approximately 2 tank volumes (120 liter) per 24 hr. An activated charcoal filter was built to remove ^{14}C-labeled compounds from the drainage water.

Organisms

The organisms used for all experiments were daphnids (Daphnia magna), snails (Helisoma sp.), algae (Oedogonium cardiacum), and mosquito fish (Gambusia affinis). An attempt to replace mosquito fish with flagfish (Jordanella floridae) failed when we were unable to rear these fish. Flagfish spawning and hatching of eggs were successful; however fry growth rate was far slower than anticipated making their use in our ecosystems impractical.

Experimental Chemicals

The following pesticides were tested in the ecosystems; the fungicide, pentachloronitrobenzene (PCNB), and herbicides, 2-chloro-4, 6-bis(ethylamino)-S-triazine(simazine) and a,a,a,-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine-(trifluralin). All these pesticides were ring ^{14}C-labeled, 43.24, 90.91, and 45.25 μCi/mg, for PCNB, simazine, and trifluraline, respectively, and the chemical purity exceeded 97%. The ^{14}C-labeled pesticides were mixed with unlabeled pesticides of equal or greater purity for the actual treatments.

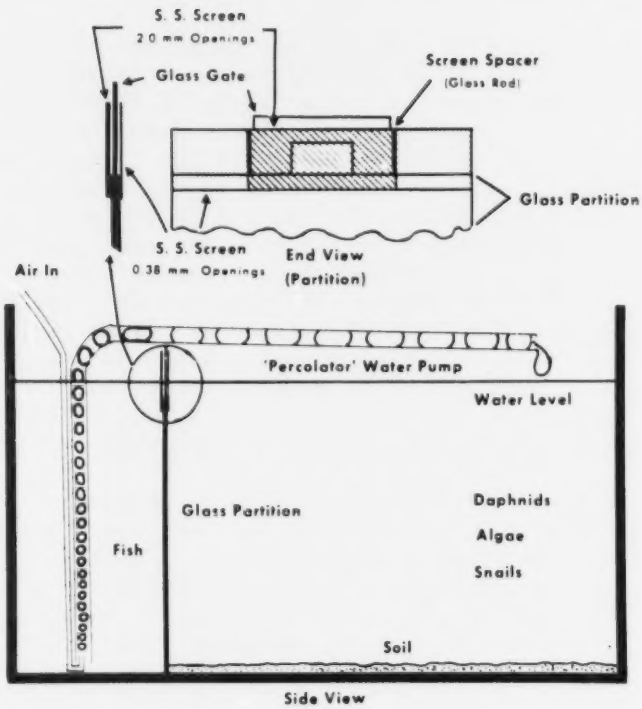

Figure 1. Recirculating static model ecosystem. Tank is 41 x 20 x 24 cm and holds 16 liters of water.

SECTION 4
EXPERIMENTAL PROCEDURES

Ecosystem Preparation

In the static systems the pesticides were adsorbed to 400 grams soil (Matapeake silt loam, pH 5.3, organic matter content 1.5%; sand, silt, and clay content of 38.4, 49.4, and 12.2%, respectively) at the rate of 1.0, 10.0, and 100.0 ppm for PCNB and trifluralin and 0.1, 1.0, and 10.0 ppm for simazine, then flooded with 16 liters of water. The amount of pesticide in water over flooded soil was found (through preliminary tests) to be about 1/1,000 of the soil concentration. Therefore, these soil rates were selected to approximate the water concentration in the flowing systems. One day after flooding 24 fish, 24 snails, about 1 gram algae and approximately 200 daphnids were added to each tank. The serial diluter was turned on to fill the tanks of the flowing system at least one week before the test chemical was introduced. During this time, the organisms were added (same number as for the static systems) and the diluter system was checked for proper operation. Metering of the test chemical through the diluter was started the same day as the organisms were introduced into the static tanks. The pesticides were introduced at the rate of 1, 10, and 100 ppb for PCNB and trifluralin and 0.1, 1.0, and 10.0 ppb for simazine.

Sampling and Analysis

The sampling times shown in Tables 1-13 represent a treatment phase followed by a desorption phase. The desorption phase was achieved by transferring the remaining fish, snails, and algae to untreated water (static systems) or by discontinuing the input of the treatment chemical in the diluter (flowing system). Two fish, two snails, about 30 mg algae and 15 to 20 mg daphnids were taken at each sampling time. The daphnid samples were analyzed by standard liquid scintillation methods. Fish and snails were homogenized in methanol and the ^{14}C-content was determined by liquid scintillation analysis. Algae samples were either combusted (PCNB experiments) or oxidized in a Packard Tricarb Oxidizer (simazine and trifluralin experiments). The $^{14}CO_2$ (from either source) was analyzed by scintillation methods. Water samples were taken for direct liquid scintillation analysis (duplicate 1-ml samples) and/or extracted with organic solvent and then analyzed. Water samples did not always coincide with tissue sampling for the PCNB and simazine experiments, but did for the trifluralin experiments. Snail and fish homogenates were spotted on silica gel TLC plates (20 x 20 cm GF-234, E. Merck, Darmstadt) and developed with the following solvent systems: PCNB, n-heptane: acetone:methanol (70:30:2 v/v/v); simazine, toluene:acetone (85:15 v/v); trifluralin, ethyl acetate:cyclohexane (1:1 v/v). Each plate was autoradiographed for 1 to 2 weeks with Kodak No. Screen medical x-ray film, NS-54T and then the labeled spots were scraped and the ^{14}C activity determined by standard liquid scintillation methods.

SECTION 5
RESULTS AND DISCUSSION

PCNB

Two PCNB experiments were conducted; the second to improve techniques and correct problems that developed in the first experiment. In the static systems, PCNB was introduced at the rate of 1 and 10 ppm (Experiment I) which resulted in very low ^{14}C water levels. Treatment rates of 10 and 100 ppm in Experiment II yielded much higher ^{14}C water levels in the water (Table 1). Numerous problems were encountered in the flowing systems, namely, mechanical malfunction of the dilution apparatus and precipitation of PCNB on glassware of the dosing system. PCNB precipitated from both carrier solutions (ethanol:Triton X-100, 7:3, Experiment I and triethylene glycol in Experiment II) when they contacted water, resulting in lower than anticipated water concentrations.

The data for the recirculating static systems is presented in Tables 2 and 3. Equilibrium water concentrations were reached in one day and remained nearly constant with time (Table 1). Similar soil treatment rates (10 ppm) resulted (with one exception) in similar water contents between the two experiments. Tissue contents of PCNB generally reflected treatment rate, while variations in tissue content with time continued to be high in both experiments. These variations are higher than those obtained in some previous studies in our laboratory using similar static systems with pesticides of similar physical-chemical properties.

The data for the flowing systems is presented in Tables 4 and 5. Water content of PCNB was much lower at the 100 ppb treatment rate of Experiment II than for Experiment I, which was undoubtedly due to the mixing chamber insolubility problems. Variations in water concentrations with time were also more variable in Experiment II. The concentration of PCNB in the four species of organisms is shown in Tables 4 and 5. Unfortunately, few sampling times coincided (samples were taken approximately 10 days apart for Experiment I and on days 1, 3, 7, 15, and 30 for Experiment II) so direct comparisons are difficult. However, a general trend of similar concentrations between the two experiments (for comparative treatments) is evident for daphnids, snails, and fish. This similarity was unexpected considering that water concentrations were different (particularly at the 100 ppb treatment level). The 100 ppb treatment rate of Experiment II was not toxic to daphnids, as compared to Experiment I, where the highest concentration prevented the establishment of a stable population. Thus, the water concentration established by chemical analysis was confirmed by biological response. The PCNB concentrations in

TABLE 1. PCNB* WATER CONCENTRATIONS IN AQUATIC MODEL ECOSYSTEMS

Experiment†	Treatment‡	Days after start of experiment								
		1	3	4	7	11	15	18	25	30
Static I	1			5.7±4.4		0		2.8±1.5	5.6±2.7	
	10			11.1±1.8		15.7±1.0		19.5±1.3	18.2±2.1	
Static II	10	2.4	2.2		11.0		1.1		1.0	
	100	10.0	7.8		7.8		7.4		4.2	
Flowing I	1			1.3±0.3		0.8±0.4		0.7±0.9	0.7±0.3	2.1±2.0
	10			5.8±5.0		5.1±1.1		2.5±0.5	5.0±0.2	4.2±0.6
	100			42.1±6.1		56.7±3.7		65.7±1.0	58.7±0.9	60.5±0.7
Flowing II	1		1.4		0.8		1.9		0.6	0.3
	10		3.2		1.0		1.8		0.5	2.9
	100		2.9		5.3		13.3		8.0	24.0

* ppb

† Experiments I and II were conducted two months apart.

‡ Concentration of PCNB adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

TABLE 2. ACCUMULATION OF ^{14}C-PCNB* BY ALGAE AND DAPHNIDS IN RECIRCULATING STATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment‡	Treatment§	Organisms	Days after start of experiment†												
			1	3	7	11	15	20	25	32	35	42	45	56	65
I	1	Algae#				1.1		0.2		0.4		0.1			
	10					0		2.6		3.5		2.2			
II	10	Algae	1.0	2.1	2.2		2.0		3.1		0.8		1.3	0.9	0.4
	100		6.2	36.1	24.9		7.4		25.6		7.4		14.6	6.8	5.0
I	1	Daphnids##				0		1.1		0.2		0			
	10					25.0	0.9	0.7		1.1		0.1			
II	10	Daphnids		6.8	3.8		6.8		3.2						
	100			43.5	35.0		70.2		30.7		17.3		16.6		

* Tissue concentration (ppm) based on total ^{14}C analysis (PCNB plus metabolites).

† A desorption phase was started after day 32 (expt. I) and day 42 (expt. II) by placing organisms in untreated water.

‡ Experiment I and II were conducted two months apart.

§ Concentration (ppm) of PCNB adsorbed to 400 grams soil.

Oedogonium cardiacum.

Daphnia magna.

TABLE 3. ACCUMULATION OF ^{14}C-PCNB* BY FISH AND SNAILS IN RECIRCULATING STATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment‡	Treatment§	Organism	Days after start of experiment†												
			1	3	8	11	15	20	25	32	35	36	42	45	56
I	1	Fish#				1.3±0.4		0.6±0.4	3.8±0.2	0.2±0.1		0	0		
	10					10.5±0.2		6.7±0.8	10.9	12.6±2.4		1.5	0.3		
II	10	Fish	0.2±0	0.2±0	3.5±0.2		2.8±1.9		1.7±0.6					2.0±0.7	0.2±0.2
	100		1.2±0.1	1.1±0.1	18.5±3.8		14.9±1.0		16.4±3.9					18.2±2.5	9.6±6.0
I	1	Snails##					2.3±1.1			2.0±1.3			0		
	10									8.4±2.7			0		
II	10	Snails		3.7±1.1	4.6±4.1						0.6±0.4			1.7±1.4	0.3±0.1
	100			44.0±11.5	14.0±4.4						5.8±1.9			5.6±2.5	1.2±0.3

* Tissue concentration (ppm) based on total ^{14}C analysis (PCNB plus metabolites).

† A desorption phase was started after day 32 (expt. I) and day 42 (expt. II) by placing organisms in untreated water.

‡ Experiment I and II were conducted two months apart.

§ Concentration (ppm) of PCNB adsorbed to 400 grams soil.

Gambusia affinis.

Hellsomi sp.

** Standard deviation.

TABLE 4. ACCUMULATION OF ^{14}C-PCNB* BY DAPHNIDS AND ALGAE IN FLOWING MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment‡	Treatment§	Organism	Days after start of experiment†									
			1	3	7	11	15	20	25	30	32	42
I	1	Daphnids#				0.6		0.4±0.3		0.6		0
	10					2.1±0.3##		1.3±0.1		2.3±1.4		3.2±1.0
	100											
II	1	Daphnids	0.6±0.8	0.2±0.3	2.3		0.5±0.6			0.6±0.1		
	10		0.9±0.6	0.5±0.6	7.0±1.7		6.4±4.1			3.3±1.6		
	100		8.6±2.9	4.4±2.2	72.9±17.2		113.6±72.2					
I	1	Algae**				0		0.4			0.2	0
	10					7.2±2.2		4.0±0.6			4.4±0.2	0.8
	100					76.4±22.9		82.5±15.6			77.2±6.2	53.1±13.2
II	1	Algae	0.1	0	0.1		0.75±0.75		2.0±0.5	1.0		0.1±0
	10		0.2±0.2	1.6	2.4±1.6		360.4±434		9.4±5.4	1.8±0.8		0.3±0.1
	100		2.3±0.5	6.7	127.7±91.4		142.7±156.4		472.0±608	164.5±85.0		11.3±8.6

* Tissue concentration (ppm) based on total ^{14}C analysis (PCNB plus metabolites).

† A desorption phase was started after day 30 by turning off the dosing apparatus in the serial diluter.

‡ Experiment I and II were conducted two months apart.

§ Theoretical water concentration of PCNB in ppb.

Daphnia magna.

Standard deviation.

** *Oedogonium cardiacum*.

TABLE 5. ACCUMULATION OF ^{14}C-PCNB* BY SNAILS AND FISH IN FLOWING MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment‡	Treatment§	Organism	Days after start of experiment†									
			1	3	7	11	15	20	25	30	32	42
I	1	Snail#					0.1	0.8±0.9			0.2	0.1±0.1
	10						5.7±2.4##	5.8±2.4			5.5±4.8	0.3±0.2
	100						48.2±29.7##	82.2±22.3			79.2±12.6	3.6±1.0
II	1	Snail		2.4±2.3	4.4					0.2±0.1		0.5±0.8
	10			2.4±2.3	0.9±0.5					13.4±13.2		5.0±4.5
	100			7.8±0.9	28.1±10.0					101.9±2.7		59.4±19.7
I	1	Fish**				0.2	0.9	1.3±0.9	0.4		1.2±1.9	0
	10					1.6±1.6	3.6±0.5	4.2±0.9	3.5±0.6		5.0±3.8	0
	100					63.1±30.1	59.9±0.6	64.7±12.8	98.2±19.2		99.4±35.2	10.8±0.6
II	1	Fish	0.8±1.0	0.3±0.4	0.7±0.5		0.6±0.2		0.4±0.3	0.5±0.2		0.1±0.1
	10		0.5±0.7	0.2±0.3	2.4±1.0		4.3±0.8		5.3±1.0	4.1±2.1		0.7±0.3
	100		5.6±1.8	2.7±1.7	26.6±11.7		52.0±15.0		75.0±12.2	88.4±22.0		23.9±4.2

* Tissue concentration (ppm) based on total ^{14}C analysis (PCNB plus metabolites).

† A desorption phase was started after day 30 by turning off the dosing apparatus in the serial diluter.

‡ Experiment I and II were conducted two months apart.

§ Theoretical water concentration of PCNB in ppb.

\# Hellsoma sp.

\#\# Standard deviation.

** Gambusia affinia.

algae were very high and erratic for Experiment II, possibly due to the mats of floating algae directly receiving treatment water. Any undissolved PCNB would then be directly deposited on the algae, resulting in unusually high "accumulation" rates.

Organisms in the static systems appeared to reach equilibrium concentrations faster than in the flowing systems. However, at similar water concentrations, tissue content for daphnids, snails and fish were reasonably close.

Thin layer chromatographic analysis of snails and fish revealed that considerable metabolism of PCNB had occurred, but the number of metabolites and amount of each was nearly the same for both the static and flowing systems.

Simazine

Lower soil application rates were used for the static system (0.1, 1.0 and 10.0 ppm) to avoid possible toxicity to the algae. In the flowing system, simazine was dissolved in an ethanol:water mixture (1:13) and metered into the mixing chamber with a peristaltic pump. Dosing rates of 0.1, 1.0 and 10.0 ppb were used. Water contents shown in Table 6 reflect a dosing apparatus malfunction on day one, which resulted in a high simazine input to 1.0 ppm tanks.

The amount of simazine accumulated by the various organisms is presented in Tables 7 and 8. For the flowing system (day one) all organisms contained much more simazine at the 1.0 ppb rate than at the 10.0 ppb rate, reflecting the dosing apparatus malfunction. The simazine in these organisms desorbed rapidly as the water concentration declined to normal levels. The organisms in the static systems generally reached an equilibrium tissue concentration by day one and did not accumulate appreciable amounts of simazine after that day although a slight increase in tissue level did occur.

The snail tissue levels do not appear to be significantly different between the two systems, which may reflect the snail feeding habits and the simazine present on the various substrates. The algae and daphnia show higher tissue levels of simazine in the continuous dosing system than those found in the recirculating static, possibly reflecting the larger amount of simazine available during the thirty days. The concentration of simazine in fish (static system) increased with water level and did not change much over time. In the flowing system, however, tissue content in fish was very erratic, both between treatment rates and with time. We know of no explanation for these results.

Most of the organisms readily lost simazine when placed in untreated water (static) or when the dosing apparatus was turned off (flowing). The rate of simazine loss was somewhat slower in the flowing system probably due to residual simazine desorbing from tank surfaces.

TABLE 6. SIMAZINE WATER CONCENTRATIONS* IN AQUATIC MODEL ECOSYSTEMS

Experiment	Treatment†	Day of test		
		1	3	7
Static	0.1	0.7±0	0.9±0	1.4±0.1
	1.0	8.1±0.8	10.4±0.4	14.2±0.2
	10.0	100.0±3.8	137.3±5.5	160.5±17.8
Flowing	1.0	10.2±4.8	1.0±0.2	1.1±0.3
	10.0	43.8±3.4‡	5.7±0.6	7.2±0.8
	100.0	5.7±5.4‡	55.1±1.0	56.3±1.8

* ppb.

† Concentration of simazine adsorbed to 400 gram soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ Diluter malfunction.

TABLE 7. ACCUMULATION OF ^{14}C-SIMAZINE BY DAPHNIDS AND ALGAE IN AQUATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Organism	Experiment	Treatment*	Days after start of experiment						
			1	3	7	15	30	35†	42
Daphnids‡	Static	0.1	0§	0 (0)#	0.1 (7)	0.01	0		
		1.0	0.01	0.01 (1)	0.03 (2)	0.02	0.02		
		10.0	0.21	0.20 (1)	0.21 (1)	0.22	0.31		
	Flowing	0.1	0.19	0.19 (32)	0 (0)	0.13	0.41		
		1.0	0.35	0.09 (53)	0.14 (19)	0.17	1.09		
		10.0	0.23	0.19 (4)	0.17 (3)	0.81	3.11		
Algae##	Static	0.1	0	0 (6)	0.01 (4)	0.01	0.01±0.01	0	0
		1.0	0.02±0.07	0.04±0.02 (4)	0.03±0.01 (2)	0.06±0.02	0.11±0.01	0.05±0.02	0.05±0.02
		10.0	0.19±0.09	0.35±0.05 (3)	0.46±0.08 (3)	0.38±0.06	0.91±0.12	0.40±0.04	0.75±0.39
	Flowing	0.1	0	0 (3)	0.20±0.19 (182)	0.09±0.08	0.04±0.07	0.04±0.04	0
		1.0	0.10	0.02±0.03 (9)	0.18±0.25 (25)	0.22±0.06	0.28±0.06	0.06±0.06	0.07
		10.0	0.06	0.23±0.03 (4)	1.15±0.39 (20)	1.72±0.64	1.54±0.44	1.27±0.31	0.92±0.46

* Concentration of simazine adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into its water (flowing, ppb).

† After day 30 the organisms were allowed to desorb in untreated water.

‡ *Daphnia magna*.

§ Tissue concentration (ppm) based on total ^{14}C analysis (simazine plus metabolites).

Bioaccumulation ratios (in parenthesis) = tissue concentration/water concentration.

Oedogonium cardiacum.

TABLE 8. ACCUMULATION OF ^{14}C-SIMAZINE* BY FISH AND SNAILS IN AQUATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Organism	Experiment	Treatment†	Days after start of experiment						
			1	3	7	15	30	35‡	42
Fish§	Static	0.1	0.01±0.02#	0.04±0.04 (50)##	0.06±0.06 (44)	0.12±0.06	0.11±0.07	0.03±0.03	0
		1.0	0.25±0.08	0.20±0.03 (19)	0.45±0.13 (32)	1.22±0.75	0.36±0.04	0.09±0.01	0.03
		10.0	2.98±1.69	2.94±0.73	2.61±0.05	4.61±0.88	4.51±0.84	2.00±0.82	0.09
	Flowing	0.1	2.07±0.69	2.71±3.70 (435)	1.64±2.76 (1477)	0.03±0.05	0.01±0.02	0.04±0.05	0.01
		1.0	2.74±0.94	11.50±6.25 (676)	2.58±3.76 (354)	0	0.71±0.41	0.86±1.46	0.12
		10.0	2.21±1.66	0.23±0.22	0.68±1.11	1.21±1.19	1.60±2.41	0.27±0.07	0.02
Snails**	Static	0.1	0	0 (0)	0 (7)	0	0	0	0
		1.0	0.01±0.01	0.01±0 (0)	0.01±0.01 (12)	0.02±0.02	0.02±0	0.01	0
		10.0	0.06±0.01	0.13±0.03 (1)	0.22±0.05 (2)	0.21±0.03	0.23±0.04	0.09	0.04
	Flowing	0.1	0.65±0.92	0.02±0.02 (3)	0.01±0.02 (0)	0.05±0.05	0.01±0.01	0.06	0.01
		1.0	1.21±1.67	0.11±0.16 (63)	0.17±0.08 (1)	0.01±0.02	0.06±0.07	0.03	0.07
		10.0	0.40±0.62	0.04±0.04 (1)	0.28±0.07 (4)	0.23±0.09	0.16±0.06	0.13	0.07

* Tissue concentration (ppm) based on total ^{14}C analysis (simazine plus metabolites).

† Concentration of simazine adsorbed to 400 gram soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ After day 30 the organisms were placed in untreated water.

§ Gambusia affinis.

Standard deviation.

Bioaccumulation ratio = tissue concentration/water concentration.

** Helisoma sp.

Thin layer chromatographic analysis of fish and snail extracts indicated that different breakdown processes were occurring in the two systems. In the fish homogenates from the static and flowing systems, 30-70% and 8-16%, respectively, of the total ^{14}C was hydroxysimazine. In the flowing system, a major unidentified metabolite, which moved near the solvent front, accounted for the rest of the ^{14}C. The snails contained both the unidentified metabolite and hydroxysimazine in the same proportion in both systems.

Trifluralin

The concentration of trifluralin in water reflected its treatment rate for both the static and flowing systems (Table 9). These values are based on extraction analysis of the water (ethyl acetate:hexane, 70:30). The direct count analysis of water on day 15 and 30, highest rates only, indicate that much of the ^{14}C in the static system was not trifluralin, while most of the ^{14}C in the flowing system was.

Significantly different bioaccumulation ratios (BR) were observed between the static and the continuous dosing systems (Tables 10-13). Ratios were generally much lower for the static systems and were similar to the values obtained from a previous study on the distribution of dinitroaniline herbicides in the aquatic environment (Kearney, et al. 1977). Bioaccumulation ratios of several thousand were obtained with the continuous dosing system; values which are sufficiently high to indicate a potential environmental risk. Differences in the design of the two ecosystems and the degradation characteristics of trifluralin largely explain the variation in results. In the static system, trifluralin in solution would degrade within a few days, leaving more polar products in solution. These degradation products would be accumulated less by the organisms than the unaltered trifluralin. However, it should be pointed out that the BR were based on total ^{14}C in tissue and extracted water. Therefore, in the static system, the actual ratios between trifluralin in water and tissue may have been about the same as for the flowing system, but the concentration of trifluralin in water and tissue would have been much lower and changing with time. In the continuous dosing system, organisms were exposed to a continuous supply of unaltered trifluralin, which would allow for maximum accumulation to occur. The results support this concept since the highest BR values for the static systems were obtained on day 1 and 3 then decreased with time, whereas for the continuous dosing systems, BR values increased with time. The tlc data also supports this idea; the fish and snails from the static system contained little trifluralin and several metabolites while the tissue samples from the continuous dosing systems contained almost entirely trifluralin.

The effects data also illustrated the differences in the two systems. No noticeable effects were observed in the static systems, while in the continuous dosing systems (at the high rates) fish were observed with yellow bellies, unusual swimming behavior, (i.e. swimming upside down, laying upside down on the bottom, jerking, general inability to maintain a proper orientation in the water column) darkening of the tail pigmentation, and what appeared to be a

TABLE 9. TRIFLURALIN WATER CONCENTRATIONS* IN AQUATIC MODEL ECOSYSTEMS

Experiment	Treatment†	Day of test				
		1	3	7	15	30
Static	1	0.23	0.70	0.40	0.83	0.86
	10	3.40	2.50	2.87	8.37	9.10
	100	36.93	31.33	55.10	148.80‡ (223.92)	160.10 (337.77)
Flowing	1	0.12	0.17	0.20	0.45	0.83
	10	0.47	0.91	1.40	2.58	2.48
	100	9.27	16.23	19.60	29.83 (25.33)	21.62 (21.73)

* ppb.

† Concentration of trifluralin adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ Values in parenthesis are one ml direct counts others are 50 ml extraction values.

TABLE 10. ACCUMULATION OF ^{14}C-TRIFLURALIN* BY DAPHNIA MAGNA IN RECIRCULATING STATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Treatment†	Days after start of experiment				
	1	3	7	15	30
1	0.15+0.06‡ (617)§	0.05+0.07 (84)	0.49+0.25 (1293)	0 (0)	0.08+0.11 (69)
10	1.9+0.27 (576)	2.69+0.18 (1107)	0.84+0.11 (300)	0.22+0.11 (27)	0.43+0.0 (47)
100	19.55+0.80 (536)	19.64+1.9 (628)	13.99+2.44 (254)	6.40+0.93 (43)	5.19+0.20 (33)

* Tissue concentration (ppm) based on total ^{14}C analysis (trifluralin plus metabolites).

† Concentration of trifluralin (ppm) adsorbed to 400 grams soil.

‡ Standard deviation.

§ Bioaccumulation ratio = tissue concentration/water concentration in parenthesis).

TABLE 11. ACCUMULATION OF ^{14}C-TRIFLURALIN* BY OEDOGONIUM CARDIACUM IN MODEL AQUATIC ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment	Treatment†	Days after start of experiment						
		1	3	7	15	30	35‡	50
Static	1	0.17±0.27§ (775)#	0.15±0.18 (589)	---	0.15±0.18 (175)	0.03±0.06 (190)	0.24±0.13	0.14±0.22
	10	1.65±1.09 (377)	0.56±0.17 (170)	0.70±0.40 (281)	1.83±1.03 (218)	2.12±0.80 (247)	1.24±0.91	0.90±0.36
	100	38.05±15.12 (791)	5.05±0.93 (138)	15.23±2.87 (276)	31.56±6.06 (210)	35.38±4.11 (147)	25.43±7.87	16.64±5.45
Flowing	1	0.04±0.08 (250)	0.90±1.45 (5294)	3.98±8.44 (34811)	1.26±1.28 (4480)	1.46±1.30 (1929)	0.55±0.42	12.50±22.75
	10	0.11±0.12 (474)	1.38±1.71 (1467)	33.12±22.85 (24678)	11.07±6.92 (5760)	3.11±2.13 (1983)	2.70±0.76	38.43±65.25
	100	2.61±0.55 (196)	41.47±5.23 (2553)	90.79±32.42 (5062)	112.30±88.33 (3858)	102.15±78.76 (3961)	26.65±12.17	7.82±3.18

* Tissue concentration (ppm) based on total ^{14}C analysis (trifluralin plus metabolites).

† Concentration of trifluralin adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ After day 30 the organisms were placed in untreated water.

§ Standard deviation.

Bioaccumulation ratio = tissue concentration/water concentration.

TABLE 12. ACCUMULATION OF ^{14}C-TRIFLURALIN* BY HELISAOMA sp. IN AQUATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment	Treatment†	Days after start of experiment						
		1	3	7	15	30	35‡	50
Static	1	0.23±0.06§ (1067)#	0.09±0.07 (361)	0 (0)	0.01±0.02 (0)	0.03±0.04 (23)	0.06±0.05	0.01±0.04
	10	0.49±0.29 (149)	0.53±0.45 (161)	0.02±0.03 (6)	0.27±0.21 (32)	0.12±0.13 (13)	0.09±0.05	0.03±0.04
	100	5.57±2.14 (155)	4.42±2.62 (125)	2.39±0.83 (43)	3.46±0.82 (23)	3.73±1.76 (19)	2.09±0.96	0.94±0.28
Flowing	1	0.15±0.14 (1250)	0.14±0.27 (824)	0.22±0.40 (1260)	0.33±0.26 (1017)	0.12±0.29 (19)	0.28±0.18	0.17±0.08
	10	0.43±0.22 (1583)	0.07±0.48 (752)	3.30±5.29 (2285)	4.85±5.34 (2718)	1.54±2.32 (535)	0.63±0.33	0.31±0.09
	100	2.45±2.40 (260)	17.99±17.07 (1087)	31.07±21.72 (1494)	32.52±16.66 (1085)	18.74±12.37 (878)	4.89±31.47	4.30±2.01

* Tissue concentration (ppm) based on total ^{14}C analysis (trifluralin plus metabolites).

† Concentration of trifluralin adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ After day 30 the organisms were placed in untreated water.

§ Standard deviation.

Bioaccumulation ratio = tissue concentration/water concentration.

TABLE 13. ACCUMULATION OF ^{14}C-TRIFLURALIN* BY GAMBUSIA AFFINIS IN AQUATIC MODEL ECOSYSTEMS AS INFLUENCED BY TIME AND TREATMENT

Experiment	Treatment†	Days after start of experiment						
		1	3	7	15	30	35‡	50
Static	1	0.15±0.33§ (1184)#	2.21±2.23 (4495)	0.26±0.42 (647)	4.57±4.48 (6191)	2.14±1.50 (2583)	0.69±0.74	0.17±0.03
	10	1.12±0.88 (340)	2.68±0.84 (1042)	1.05±0.89 (496)	4.16±5.27 (493)	2.67±3.22 (296)	0.39±0.25	0.50±0.50
	100	25.37±3.82 (686)	35.95±5.34 (1167)	19.18±5.59 (345)	11.63±1.49 (78)	10.74±2.43 (68)	4.08±1.33	1.83±1.69
Flowing	1	1.05±1.04 (8750)	0.56±0.45 (3294)	1.22±1.62 (5969)	0.91±0.93 (1668)	2.58±2.61 (3036)	1.85±1.37	0.72±0.65
	10	1.07±1.15 (3591)	2.39±1.91 (2576)	7.99±1.12 (5706)	5.43±1.98 (2344)	12.72±15.24 (6709)	2.07±1.00	1.02±0.59
	100	11.10±1.64 (1211)	30.94±7.68 (1897)	77.65±25.38 (3914)	120.59±23.96 (4069)	82.29±19.46 (3919)	31.20±9.04	---

* Tissue concentration (ppm) based on total ^{14}C analysis (trifluralin plus metabolites).

† Concentration of trifluralin adsorbed to 400 grams soil (static, ppm) and theoretical concentration introduced into water (flowing, ppb).

‡ After day 30 the organisms were placed in untreated water.

§ Standard deviation.

Bioaccumulation ratio = tissue concentration/water concentration.

broken back (Figures 3 and 4). The possibility that some of these effects may be related to the presence of ethanol was not ruled out, however, previous experiments in which ethanol was used at this concentration did not produce these effects.

At the start of the experiment pregnant mosquito fish were placed in egg laying chambers within the continuous dosing test chambers. This was an attempt to determine the feasibility of using mosquito fish to obtain information on biological effects in this system. At day thirty the adults were harvested and the fry left in their respective chambers with untreated water passing through. Sixty days later, after the fry had grown to near maturity, they were harvested and analyzed.

Fry were born in all tanks except one control and one of the 100 ppb tanks. The average trifluralin content of these fish was 0.13, 0.27, and 5.03 ppm for the 1.0, 10.0 and 100.0 ppb treatment rates, respectively. Analysis of the 100 ppb treatment fish by tlc indicated that 80% of the ^{14}C was trifluralin. These results indicate that trifluralin is slowly metabolized and/or lost from fish.

The testing of three pesticides in the two model ecosystems has demonstrated their relative utility, strengths, and weaknesses. We found that the two systems cannot be routinely substituted for each other since differences in their basic design can result in very different results for the same compound (trifluralin in this study). The degradability of the test compound must be considered in choosing between these two designs. Compounds that degrade slowly and are therefore persistent in the environment would likely behave similarly (accumulation rates, total amounts accumulated and effects on organisms) in the two model ecosystems (PCNB and simazine in this study). On the other hand, compounds that degrade faster (half life 2 weeks to 2 months) would likely behave differently between the two systems. Degradation products would continuously be swept out of the flowing system so organisms would be exposed mostly to the parent compound. In the static systems, the amount of parent compound present in water would decrease with time as the amount and number of degradation products increased. Thus, with degradable pesticides, aquatic organisms would be exposed to a changing mixture of compounds in the static system and primarily to the parent compound in the flowing system. The response of the organisms would then depend on the relative effect of the parent compounds vs the metabolites on the organisms. For the static system, the theoretical ratio between the concentration of parent compound in water and the concentrate in organism, at any one time, should be about the same as the ratio obtained in the flowing system. However, the determination of that ratio in the static system is far more difficult due to the changing concentrations and presence of other degradation products.

Another major factor dictating the choice of systems is the likely mode of introduction into the environment. The static ecosystem simulates an erosional or sediment source of compounds, such as when a rainfall event causes runoff from an agricultural area recently treated with pesticides. Under these conditions pesticides would be transported into water adsorbed to suspended

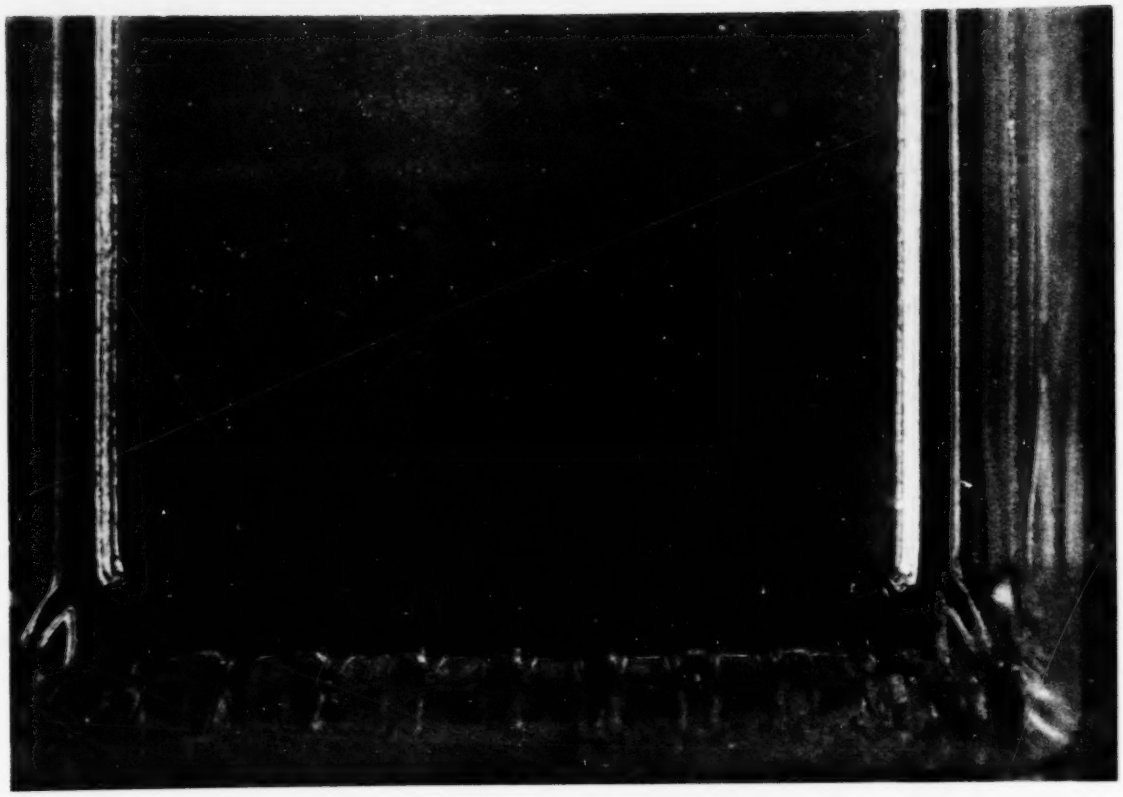

Figure 3. Adult ♂ *Gambusia affinis* with bent spine.

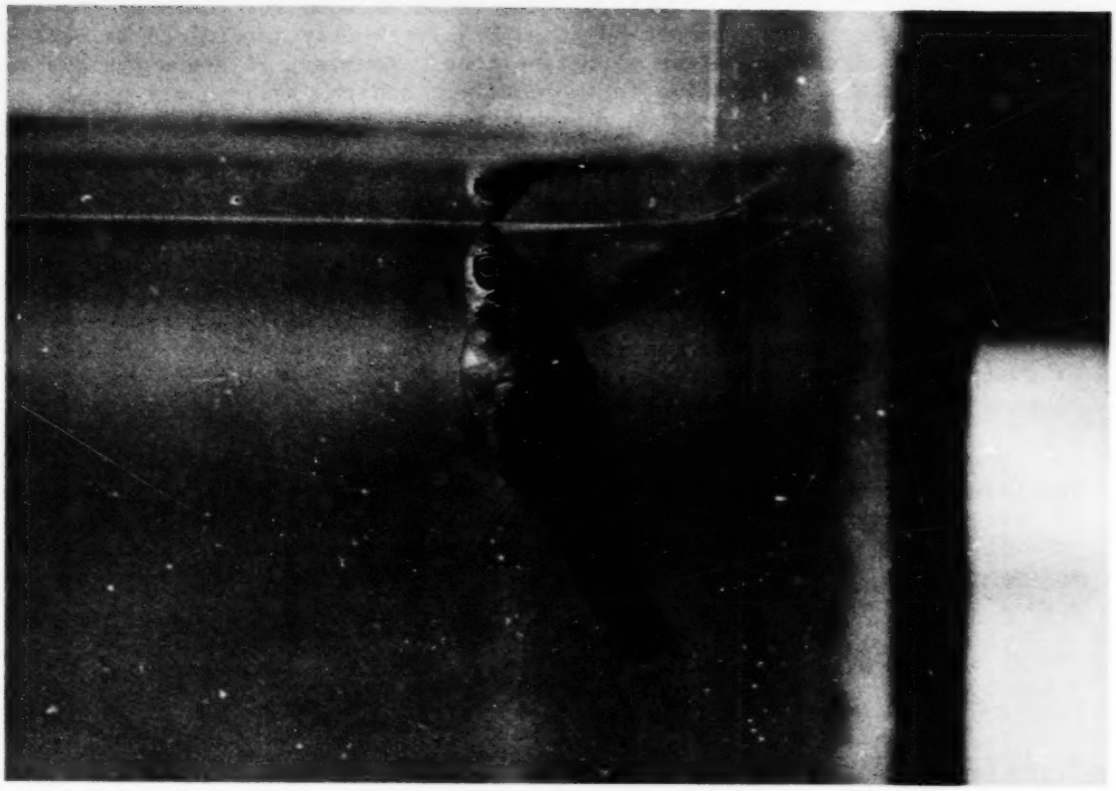

Figure 4. Adult $\overset{0}{+}$ Gambusia affinis in vertical swimming position.

soil particles. The flowing ecosystem simulates an effluent source of compounds such as industrial or manufacturing effluent, where the supply is reasonably constant and directly introduced into a waterway.

Chronic toxicity and effects studies are generally better run in flowing as compared to the static systems. The principal reason is that the identity and concentration of the toxicant is more clearly defined and controlled in the flowing system. This is primarily true for degradable compounds. The toxicant concentration would remain constant with time in the flowing system but would change rapidly in static. Static systems could be used for toxicity testing if the concentration of the parent compound was frequently determined as well as the identity and concentration of metabolites. However, it seems obvious that such an approach would require far more effort and the results may not be as reliable (due to the presence of metabolites) as the flowing system. On the other hand, for less degradable compounds and acute toxicity tests, the static system could function as well or better than the flowing system.

The cost and supply of radiolabeled compounds can also influence the choice between the static or flowing systems. Use of radiolabeled compounds in ecosystem research is indispensable if the identity and concentration of degradation products is desired. In our studies the flowing systems used at least 10 times more labeled material than did the static systems. Therefore, if there is a limited supply of labeled compound for a study, the use of the flowing system may be in question.

The daphnid population was found to be more stable in the static than in the flowing system primarily because the food supply was better. An addition of "green water" to the flowing tanks improved the daphnid population considerably. Therefore, the addition of food to the flowing systems, possibly through the continuous dosing system, would probably increase the daphnid population to near static system levels.

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